
Citation:

O'Hara, JP and Duckworth, L and Black, A and Woods, D and Mellor, A and Boos, C and Gallagher, L and Arjomandkhah, N and Morrison, D and Preston, T and King, R (2019) Fuel Use during Exercise at Altitude in Women with Glucose–Fructose Ingestion. *Medicine and Science in Sports and Exercise*. ISSN 1530-0315 DOI: <https://doi.org/10.1249/MSS.0000000000002072>

Link to Leeds Beckett Repository record:

<https://eprints.leedsbeckett.ac.uk/id/eprint/5992/>

Document Version:

Article (Accepted Version)

The aim of the Leeds Beckett Repository is to provide open access to our research, as required by funder policies and permitted by publishers and copyright law.

The Leeds Beckett repository holds a wide range of publications, each of which has been checked for copyright and the relevant embargo period has been applied by the Research Services team.

We operate on a standard take-down policy. If you are the author or publisher of an output and you would like it removed from the repository, please [contact us](#) and we will investigate on a case-by-case basis.

Each thesis in the repository has been cleared where necessary by the author for third party copyright. If you would like a thesis to be removed from the repository or believe there is an issue with copyright, please contact us on openaccess@leedsbeckett.ac.uk and we will investigate on a case-by-case basis.

Fuel Use during Exercise at Altitude in Women with Glucose–Fructose Ingestion

John P O'Hara¹, Lauren Duckworth¹, Alistair Black¹, David R Woods^{1,2,3}, Adrian Mellor^{1,2,4}, Christopher Boos⁵, Liam Gallagher¹, Costas Tsakirides¹, Nicola C. Arjomandkhah⁶, Douglas J Morrison⁷, Thomas Preston⁷, Roderick FGJ King¹

¹ Research Institute for Sport, Physical Activity and Leisure, Leeds Beckett University, Leeds, UK

² Royal Centre for Defence Medicine, Birmingham, UK

³ Northumbria NHS Trust and Newcastle Trust, UK

⁴ James Cook University Hospital, Middlesbrough, UK

⁵ Poole Hospital, Department of Cardiology, Poole, Dorset, UK

⁶ School of Social and Health Sciences, Leeds Trinity University, Leeds, UK.

⁷ Scottish Universities Environmental Research Centre, University of Glasgow, East Kilbridge, UK

Short Title: Substrate oxidation in women at high altitude

Address for Correspondence:

John O'Hara, Research Institute for Sport, Physical Activity and Leisure, School of Sport, Leeds Beckett University, Headingley Campus, Leeds, LS6 3QS, UK.

Tel: +44 (0) 113 8125239

Email: J.OHara@leedsbeckett.ac.uk

Abstract

Purpose: This study compared the co-ingestion of glucose and fructose on exogenous and endogenous substrate oxidation during prolonged exercise at terrestrial high altitude (HA) versus sea level, in women. **Method:** Five women completed two bouts of cycling at the same relative workload (55% W_{max}) for 120 minutes on acute exposure to HA (3375m) and at sea level (~113m). In each trial, participants ingested $1.2 \text{ g} \cdot \text{min}^{-1}$ of glucose (enriched with ^{13}C glucose) and $0.6 \text{ g} \cdot \text{min}^{-1}$ of fructose (enriched with ^{13}C fructose) before and every 15 minutes during exercise. Indirect calorimetry and isotope ratio mass spectrometry were used to calculate fat oxidation, total and exogenous carbohydrate oxidation, plasma glucose oxidation and endogenous glucose oxidation derived from liver and muscle glycogen. **Results:** The rates and absolute contribution of exogenous carbohydrate oxidation was significantly lower at HA compared with sea level ($ES > 0.99$, $P < 0.024$), with the relative exogenous carbohydrate contribution approaching significance (32.6 ± 6.1 vs. $36.0 \pm 6.1\%$, $ES = 0.56$, $P = 0.059$) during the second hour of exercise. In comparison, no significant differences were observed between HA and sea level for the relative and absolute contributions of liver glucose (3.2 ± 1.2 vs. $3.1 \pm 0.8\%$, $ES = 0.09$, $P = 0.635$ and 5.1 ± 1.8 vs. 5.4 ± 1.7 grams, $ES = 0.19$, $P = 0.217$), and muscle glycogen ($14.4 \pm 12.2\%$ vs. $15.8 \pm 9.3\%$, $ES = 0.11$, $P = 0.934$ and 23.1 ± 19.0 vs. 28.7 ± 17.8 grams, $ES = 0.30$, $P = 0.367$). Furthermore, there was no significant difference in total fat oxidation between HA and sea level (66.3 ± 21.4 vs. 59.6 ± 7.7 grams, $ES = 0.32$, $P = 0.557$). **Conclusion:** In women, acute exposure to HA reduces the reliance on exogenous carbohydrate oxidation during cycling at the same relative exercise intensity.

Keys Words: Acute Hypoxia, Carbon Isotope, Exogenous Carbohydrate Oxidation, Liver Glycogen, Muscle Glycogen, Plasma Glucose Oxidation

1 Introduction

2
3 Endogenous stores of carbohydrate and fat are utilized as substrates to meet the metabolic demands
4 of the working muscle, as well as exogenous sources of carbohydrate if provided. The contribution
5 of carbohydrate and fat oxidation to energy expenditure at sea level is primarily related to the
6 intensity and duration of exercise, as well as the type and amount of carbohydrate ingested.
7 However, exposure to high altitude (HA) and the reduction in arterial oxygen saturation, is likely
8 to alter substrate oxidation during exercise compared with sea level. The complete oxidation of
9 carbohydrate requires less oxygen per mole of ATP synthesized compared with the oxidation of
10 free fatty acids (1) potentially leading to a greater reliance on carbohydrate oxidation in hypoxia.
11 However, the literature is inconclusive when comparing substrate oxidation during exercise
12 between hypoxia and normoxia (2). This heterogeneity may be explained by variations in study
13 design, such as participants' sex, method of exercise intensity determination (absolute vs. relative)
14 and whether carbohydrate was ingested or not (2).

15
16 Greater dependency on plasma glucose has been shown in men when comparing acute hypoxia
17 with normoxia, using the same absolute exercise intensity (3, 4). Conversely, when the exercise
18 intensity is normalized to the same relative exercise intensity the literature for men is equivocal
19 (2). These data either show an increase (5, 6) or no change in the respiratory exchange ratio (RER)
20 (7, 8). When an exogenous source of carbohydrate is provided on acute exposure to HA, men have
21 shown an increased reliance on endogenous carbohydrate oxidation (9), as well as a reduced
22 reliance on muscle glycogen and increased fat oxidation (10) during exercise at the same relative
23 intensity. However, it is known that sex influences substrate oxidation during exercise at sea level,
24 with women generally relying more on fat than carbohydrate as a fuel source compared with men

(11, 12). This has been attributed to women having a higher percentage of body fat (12), a greater lipid content in muscles fibers (11) and better mobilization of fatty acids from subcutaneous adipose tissue linked to cyclic changes in estrogen and progesterone (13). However, following the provision of an exogenous source of glucose during exercise at sea level, most studies show no statistical sex-differences in exogenous carbohydrate oxidation during exercise (14, 15) with one exception (16). In the study by Riddell et al. (16), higher exogenous carbohydrate oxidation was accompanied by a greater reduction in endogenous carbohydrate oxidation during exercise in women compared with men. This is in contrast to other studies, which showed no difference (17) or smaller reductions in endogenous or carbohydrate oxidation compared with men (14). Despite this, there have only been limited studies assessing whole body carbohydrate or fat oxidation in women at altitude.

In contrast to men, women have a lower RER, with increased free fatty acid availability when exercising at a moderate relative exercise intensity (70% of altitude-related maximal oxygen uptake ($\dot{V}O_{2\max}$)) on acute exposure to hypoxia (4,300m) compared with normoxia (18). These women also showed no differences in substrate oxidation at HA between the early-follicular and mid-luteal menstrual cycle phases when levels of estradiol and progesterone are high. Conversely, a study, which recruited men and women, showed no change for either sex in RER during acute hypoxic exposure (4100m) compared with normoxia when exercising at a matched relative exercise intensity (50% of altitude-related $\dot{V}O_{2\max}$) (19). However, whole body carbohydrate oxidation was increased at HA in both men and women following hypoxic exposure when using the same absolute exercise intensity.

Considering the potential differences in fuel use between women and men at sea level, and what has been documented for men at altitude, it is surprising that the fuel use responses to carbohydrate ingestion during exercise at acute hypoxia have yet to be established in women. Thus, the purpose of the present study was to compare the effects of co-ingesting ^{13}C glucose and ^{13}C fructose during 120 minutes of moderate intensity cycling exercise on exogenous and endogenous fuel use during acute hypoxia (terrestrial high altitude) and normoxia (sea level) in women.

Methods

Participants

Seven women from the British military were recruited for this study, providing 86% power to detect differences in the rate of exogenous carbohydrate oxidation, with an expected mean difference of $0.2 \text{ g}\cdot\text{min}^{-1}$ between high altitude and sea level, assuming a standard deviation of $0.17 \text{ g}\cdot\text{min}^{-1}$ at an alpha of 0.05. However, only five completed the study due to attrition at points where it was not possible to recruit additional participants. The five participants (age 25 ± 2 years, body mass $61.7 \pm 5.6 \text{ kg}$), were engaged in regular physical training (3-5 training days per week) and considered to be physically fit but not elite athletes. Participants provided written informed consent before the study, which was approved by the Ministry of Defence Research Ethics Committee (Protocol 412/13). This cohort of participants were part of a larger research project and the design of this study and the associated protocols have been described previously (10).

Preliminary Testing

Participants completed two maximal incremental cycle tests to volitional exhaustion to determine their individual maximal workload (W_{max} (20)) and $\dot{V}\text{O}_{2\text{max}}$ on a bicycle affixed to a bicycle trainer (Compu Trainer® Pro Lab, Racer Mate, USA), calibrated according to the manufacturer's

instructions. The first test was performed at sea level (altitude ~113m) with the second test performed a week later during acute exposure to normobaric hypoxia (FiO_2 ~13.4% (considering water vapour partial pressure (21) and daily fluctuations of barometric pressure) equivalent to 3375m (the reported altitude for the New Refuge Torino in the Italian Alps; PiO_2 95.2 mmHg). Oxygen uptake ($\dot{V}\text{O}_2$) and carbon dioxide production ($\dot{V}\text{CO}_2$) measurements were made throughout using an online gas analysis system (Metalyser, Cortex, Germany), calibrated following the manufacturer's instructions and were calculated using standard metabolic algorithms (22) using the Haldane transformation. FiO_2 and FiCO_2 were measured continuously, rather than assuming constants, thus correcting for changes in ambient conditions. This is important in a normobaric chamber where the FiO_2 is reduced to simulate a given altitude. At sea level and normobaric hypoxia participants achieved W_{max} values of 208.5 ± 34.4 W and 190.9 ± 31.3 W, and $\dot{V}\text{O}_{2\text{max}}$ values of 44.7 ± 7.1 ml.kg⁻¹.min⁻¹ and 37.4 ± 5.9 ml.kg⁻¹.min⁻¹, respectively.

Design of the Study

Following the assessment of W_{max} , participants completed two experimental cycling trials for 120 minutes at 55% W_{max} , one at terrestrial HA (barometric pressure 506.7 ± 1.4 mmHg, PiO_2 : 96.3 ± 0.3 mmHg (New Refuge Torino, Alps, Italy)), as described previously (10) and another at sea level seven weeks later. To control for menstrual cycle phases, the women were tested in either the early-follicular or mid-luteal phase, which has no effect on fuel use at HA (18). Each cycling test involved the ingestion of 1.8 g.min⁻¹ of carbohydrate (1.2 g.min⁻¹ of glucose (D-glucose, Thornton and Ross Ltd, Huddersfield, UK) and 0.6 g.min⁻¹ of fructose (D-fructose, Danisco, Oy, Otkka, Finland) at regular intervals during exercise. Stock glucose (natural $\delta^{13}\text{C}$ abundance = -32.58 ‰) and fructose (natural $\delta^{13}\text{C}$ abundance = -30.04 ‰), was enriched using 0.24g of U-¹³C₆ D-glucose

(Cambridge Isotope Laboratories, Inc, Tewksbury, MA, USA), and 0.12g of U-¹³C₆ D-fructose (Cambridge Isotope Laboratories, Inc), achieving a combined enrichment of $\delta^{13}\text{C} = +115.88 \text{ ‰}$. All $\delta^{13}\text{C}$ measurements are quoted with reference to the internationally accepted standard for carbon isotope measurements, Vienna Pee Dee Belemnite (VPDB). The ¹³C abundance of stock glucose and fructose and ¹³C enrichment of spiked glucose and fructose was determined using liquid chromatography coupled to isotope ratio mass spectrometry (LC-IRMS; Isoprime, Cheadle, UK), using L-Fucose as an isotopic internal standard as previously described (23).

Diet and physical activity before testing

Participants recorded their food intake and activity patterns during the 72 hours before the first experimental trial and were instructed to repeat the same diet and activity pattern before the subsequent trial. Participants were required to refrain from any intense and/or prolonged physical activity, alcohol or caffeine consumption in the 36 hours before each experimental trial. In addition, they were asked to refrain from ingesting carbohydrates derived from plants which utilize the C₄ photosynthetic cycle, in which there is higher natural abundance of ¹³C (e.g. maize derived sugars) for the duration of the study. This precaution ensured that background ¹³CO₂ abundance was less likely to be perturbed from oxidation of endogenous and dietary substrate stores from naturally “enriched” C₄ origin. A standardized evening meal was consumed 12 hours before each experimental trial (total 1443 kcal; 53% carbohydrate, 17% fat, 30% protein).

Experimental Trials

Each experimental trial was performed at 19 to 21°C following an overnight fast. Participants repeated their trials at the same time of day, to avoid any influence of circadian variance. On arrival

a catheter (20 gauge Introcan Safety[®], B. Braun Medical Ltd, Sheffield, UK) was inserted into an antecubital vein for regular blood sampling. After 20 minutes of acute exposure to each environmental condition, peripheral oxygen saturation (SpO₂ (Nellcor N-20, Covidien, Dublin, Ireland) was measured and resting blood samples were drawn for the analysis of plasma glucose, serum insulin, serum free fatty acids, plasma lactate, plasma metanephrine and normetanephrine concentrations, as well as plasma ¹³C glucose enrichment.

Participants then rested for 10 minutes whereby $\dot{V}O_2$ and $\dot{V}CO_2$ measurements were made using an online gas analysis system (Metalyser, Cortex, Germany). For the measurement of ¹³C/¹²C in expired CO₂, 12 ml samples of expired gas were collected in duplicate in Labco Exetainers[®] (SerCon Ltd, Crewe, UK) via a mixing chamber (Jaeger, Germany).

After a 5 minute standardized warm up, which included the calibration of the bicycle trainer (Compu Trainer Pro Lab, Racer Mate, USA), an initial bolus of the carbohydrate solution was consumed (397ml). Participants then completed 120 minutes of cycling; 5 minutes at 40% W_{max}, 5 minutes at 45% W_{max}, 5 minutes at 50% W_{max}, 105 minutes at 55% W_{max}. These workloads were calculated from participants' sea level and normobaric hypoxic W_{max} for the sea level and HA environments, respectively. Additional boluses (229ml) of the carbohydrate solution were provided every 15 minutes throughout the 120 minute exercise period. Expired gas breath samples were collected and measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ were made every 15 minutes during exercise. Samples of expired gas for ¹³CO₂ analysis were collected during the final 60 seconds of each collection period. Samples for the analysis of plasma glucose, serum insulin, serum free fatty acids and plasma lactate were drawn every 15 minutes, those for plasma ¹³C glucose enrichment were

drawn at 60, 90 and 120 minutes and those for plasma metanephrine and normetanephrine concentrations were drawn at 60 and 120 minutes. Heart rate, rating of perceived exertion (RPE) and SpO₂ was measured every 15 minutes during cycling exercise.

Analyses

Plasma and serum samples collected at HA were initially stored at -20°C until they were transported back to the United Kingdom, where they were then stored at -80°C until analysis, as per the samples collected at sea level. All samples were analysed in accordance with the procedures described previously (10).

The ¹³C/¹²C ratio in expired CO₂ was determined using isotope ratio mass spectrometry (IRMS; AP2003, GVI Instruments Ltd, Manchester, UK). The isotopic ratio ¹³C/¹²C is derived against laboratory CO₂ (itself calibrated against VPDB) from the ion beam area ratio measurements with correction of the small contribution of ¹²C¹⁶O¹⁷O at m/z 45; the Craig correction (24). The ¹³C/¹²C ratio in plasma glucose was determined using LC-IRMS as described previously (23).

Oxidation rates of total fat, total carbohydrate, endogenous carbohydrate (liver and muscle), plasma glucose and exogenous carbohydrate derived from glucose and fructose ingestion combined, were calculated by indirect calorimetry ($\dot{V}O_2$ and $\dot{V}CO_2$) and stable isotope measurements (¹³C/¹²C ratio in expired CO₂ and plasma glucose), as detailed below.

Calculations

Total CHO and fat oxidation ($\text{g}\cdot\text{min}^{-1}$) were computed from $\dot{V}O_2$ ($\text{L}\cdot\text{min}^{-1}$) and $\dot{V}CO_2$ ($\text{L}\cdot\text{min}^{-1}$) using stoichiometric equations (25), with the assumption that protein oxidation during exercise was negligible.

The isotopic enrichment of the ingested glucose and fructose, (R_{exo}), was expressed in standard $\delta^{13}\text{C}$ units (‰) relative to VPDB (26). Exogenous carbohydrate oxidation derived from the combined ingestion of glucose and fructose (G_{exo} , grams) was computed by using equation 1 (27).

$$\text{Exogenous Carbohydrate Oxidation (g}\cdot\text{min}^{-1}\text{)} = \dot{V}CO_2 [(R_{\text{exp}} - R_{\text{ref}}) / (R_{\text{exo}} - R_{\text{ref}})] / k \quad (1)$$

where $\dot{V}CO_2$ is in litres per minute, R_{exp} is the measured isotopic composition in expired CO_2 , R_{ref} is the isotopic composition of expired CO_2 at rest before exercise and carbohydrate ingestion, R_{exo} is the measured isotopic composition of the exogenous glucose and fructose ingested, and k ($0.7426 \text{ l}\cdot\text{g}^{-1}$) is the rate adjusted value for the complete oxidation of glucose (28). The use of R_{ref} from expired CO_2 at rest is typical of studies in this area of research (9, 29) as the high ^{13}C -enrichment of exogenous glucose and fructose provides a strong signal in expired CO_2 . This cancels the confounding effects of relatively small fluctuations in background enrichment of expired CO_2 seen from rest to exercise following a Western European diet (30) on the calculation of exogenous carbohydrate oxidation. Endogenous carbohydrate oxidation was calculated by subtracting exogenous carbohydrate oxidation from total carbohydrate oxidation.

Computations were made on the assumption that, in response to exercise, ^{13}C is not irreversibly lost in pools of tricarboxylic acid cycle intermediates and/or bicarbonate, and that lactate produced from either glucose or fructose is either oxidized in muscle or recycled through gluconeogenesis to be used subsequently by complete oxidation. Essentially exogenous carbohydrate oxidation is calculated irrespective of the pathway that finally produces $^{13}\text{CO}_2$ that can be measured. The calculations are based on the assumption that $^{13}\text{CO}_2$ recovery in expired gases was complete or almost complete during exercise (31). Such computation has been shown to underestimate exogenous carbohydrate oxidation rates at the beginning of exercise because of the delay between $^{13}\text{CO}_2$ production in tissues and expired $^{13}\text{CO}_2$ at the mouth (32). Based on this, exogenous carbohydrate oxidation rates are presented from 60 minutes onwards during the exercise period, where it is expected that there would be isotopic equilibrium in the tissues and at the mouth.

Using the isotopic compositions of plasma glucose (R_{glu}) the oxidation rate of plasma glucose was computed at 60, 90 and 120 minutes during exercise (equation 2 (33)).

$$\text{Plasma glucose oxidation (g}\cdot\text{min}^{-1}) = \dot{V}\text{CO}_2 [(R_{\text{exp}} - R_{\text{ref}}) / (R_{\text{glu}} - R_{\text{ref}})] / k \quad (2)$$

The oxidation rate of muscle glycogen ($\text{g}\cdot\text{min}^{-1}$), either directly or through the lactate shuttle (34), was calculated by subtracting plasma glucose oxidation from total carbohydrate oxidation. Finally, the amount of glucose released from the liver was estimated as the difference between plasma glucose and exogenous carbohydrate oxidation (33).

Statistical Analysis

GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA) was used for graph creation. Data were normally distributed (Shapiro-Wilk) and are presented as mean \pm SD. Two-way repeated measures ANOVA was used to compare differences in fuel use and blood related variables over time and between conditions. Post-hoc analysis was performed for any significant effects using paired samples t-tests. Paired t-tests were also used to compare mean differences in relative and absolute fuel use, as well as heart rate, SpO₂ and RPE between conditions. This was supported where appropriate with 95% confidence intervals. Data were evaluated using SPSS for Windows version 22 (Chicago, USA) with statistical significance determined as $P < 0.05$. Due to participant attrition potentially affecting the power of the study, we have placed greater emphasis on effect sizes (ES) in the interpretation of these data. Cohen's d effect sizes were calculated, and interpreted using a threshold scale, where 0-0.2 was considered to be a trivial effect, 0.2-0.6 a small effect, 0.6-1.2 a moderate effect, 1.2-2.0 a large effect, and >2.0 a very large effect (35).

Results

Total Carbohydrate and Fat Oxidation

There was a *small*, but non-significant difference in total energy expenditure for 2 hours of continuous cycling between HA and sea level conditions (1154.0 ± 170.5 kcal vs. 1245.3 ± 160.5 kcal, ES=0.54; $P=0.114$). *Large* and *moderate* effect sizes indicated lower absolute carbohydrate oxidation at HA compared with sea level during the first hour, second hour and for the entire 2 hours of continuous cycling, though non-significant (table 1). *Small* non-significant effects were observed for the relative contribution of carbohydrate to the total energy yield during the second

hour of exercise when comparing HA and sea level trials ($50.2 \pm 10.2\%$ vs. $54.9 \pm 6.3\%$, ES=0.46; P=0.34). Alternatively, only *small* and *trivial* effect sizes for absolute fat oxidation were observed during the exercise periods (table 1). *Small* non-significant effects were also observed for the relative contribution of fat oxidation to the total energy yield during the second hour of exercise when comparing HA and sea level trials ($49.8 \pm 10.2\%$ vs. $45.08 \pm 6.3\%$; ES =0.46; P=0.34), figure 1.

Exogenous and Endogenous Carbohydrate oxidation.

Exogenous carbohydrate oxidation rates were *moderately* lower at HA compared with sea level, during the second hour of continuous cycling, being significant at 60 (ES=0.99, P=0.003), 90 (ES=1.19, P=0.017) and 120 minutes (ES=0.99, P=0.024), figure 2a. A *small* effect was observed for the relative contribution of exogenous carbohydrate oxidation to the total energy yield between HA and sea level, which approached significance ($32.6 \pm 6.1\%$ vs. $36.0 \pm 6.1\%$, ES=0.56, P=0.059), figure 1. Further, absolute exogenous carbohydrate oxidation during the second hour of exercise, was *moderately* and significantly lower at HA compared with sea level (table 2). There was a *moderate* but non-significant effect towards lower absolute endogenous carbohydrate oxidation at HA compared with sea level for the second hour of exercise (table 2). Furthermore, the relative contribution of endogenous carbohydrate oxidation to the total energy yield was *trivial* and non-significant between HA ($17.6 \pm 13.2\%$) and sea level ($19.0 \pm 9.8\%$, ES=0.1, P=0.725).

Oxidation of plasma glucose, liver glucose and muscle glycogen

A lower rate of plasma glucose oxidation was seen at HA compared with sea level during the second hour of exercise, with *moderate* (60 minutes, ES=1.07) and *large* (90 minutes, ES=1.61

and 120 minutes, ES=1.33) but non-significant effects (P=0.113), figure 2b. In addition, the absolute contribution of plasma glucose to the total energy yield was *moderately* and significantly lower at HA compared with sea level (table 2). The rate of liver glucose oxidation produced non-significant (P=0.471) *moderate* (60 minutes, ES=0.75), *trivial* (90 minutes, ES=0.12) and *small* effects (120 minutes, ES=0.36) between conditions, figure 2c. Further, *trivial* and non-significant effects were observed for both the relative (HA: $3.2 \pm 1.2\%$ vs. sea level: $3.1 \pm 0.8\%$, ES=0.09, P=0.635, figure 1) and absolute contributions of liver glucose (table 2) to the total energy yield between conditions during the second hour of exercise. The rate of muscle glycogen oxidation produced non-significant *moderate* (60 minutes, ES=0.92, P=0.085) and *trivial* effects (90 minutes, ES=0.04, P=0.841 and 120 minutes, ES=0.15, P=0.708) between conditions, figure 2d. *Trivial* non-significant effect was observed for the relative contribution of muscle glycogen to the total energy yield for the second hour of exercise when comparing HA and sea level ($14.4 \pm 12.2\%$ vs. $15.8 \pm 9.3\%$, ES=0.11, P=0.934), figure 1. Furthermore, the absolute contributions of muscle glycogen showed a *small* non-significant effect between conditions (table 2).

Blood Biochemistry

Plasma glucose concentrations were *moderately* higher at HA compared with sea level at 90 and 120 minutes (ES=0.74 and 0.87), with the condition and time interaction approaching significance (P=0.072), figure 3a. *Moderate to large effect* sizes indicate higher plasma lactate concentrations during exercise at HA compared with sea level (ES=0.86), but failed to reach statistical significance (P=0.324), figure 3b. There were mainly *small* and non-significant effects for serum insulin responses during exercise between conditions, which approached significance (ES<0.60, P=0.07), figure 3c. Serum free fatty acid concentrations were *moderately* higher during the initial

30 minutes of exercise at HA compared with sea level ($ES > 0.69$), but there was a non-significant condition and time interaction ($P = 0.469$), figure 3d. Metanephrine concentration was *moderately* higher at HA compared with sea level at 60 minutes ($ES = 0.94$) and *largely* higher at 120 minutes ($ES = 1.40$), however, these differences only approached significance ($P > 0.075$), figure 3e. A *very large* effect for normetanephrine concentration was observed, being significantly higher at HA compared with sea level at both 60 ($ES = 2.02$, $P = 0.009$,) and 120 minutes ($ES = 2.36$, $P = 0.006$), figure 3f.

Heart Rate, Rating of Perceived Exertion and SpO₂

Table 3 shows heart rate, RPE and SpO₂ at HA and sea level during the 2 hours of cycling, as well as during the initial and last hour of cycling.

Discussion

This study, to our knowledge, is the first to compare exogenous and endogenous (liver and muscle) carbohydrate oxidation, as well as fat oxidation during matched relative intensity (55% W_{max}) cycling at terrestrial HA and sea level in women. Exogenous carbohydrate oxidation supplied as glucose and fructose made a significant contribution to the total energy yield in both conditions during the second hour of exercise. However, the primary findings are that exogenous carbohydrate oxidation was reduced in acute hypoxia compared with normoxia in these women leading to lower absolute whole body carbohydrate oxidation at HA. The latter was not associated with alterations in the use of endogenous glycogen stores.

The suppressed rate of exogenous carbohydrate oxidation at HA ($0.86 \text{ g} \cdot \text{min}^{-1}$) compared with sea level ($1.03 \text{ g} \cdot \text{min}^{-1}$) is supported by literature in men when the relative (10) and absolute (29)

exercise intensity was matched. Our previous study in men produced similar rates of exogenous carbohydrate oxidation at HA ($0.92 \text{ g}\cdot\text{min}^{-1}$) (10). In contrast to our earlier work in men (10), the differences in the rates of exogenous carbohydrate oxidation in these women was large enough for the relative and absolute contributions to the total energy yield to demonstrate the same pattern of response, being lower at acute hypoxia compared with sea level. These differences in the absolute and relative exogenous carbohydrate responses between men and women who took part in an identical study remains to be explained. Young et al. (29) reported suppressed exogenous glucose oxidation in men, *albeit* when the absolute exercise intensity was matched between HA ($0.19 \text{ g}\cdot\text{min}^{-1}$) and sea level ($0.38 \text{ g}\cdot\text{min}^{-1}$). However, their rates were lower compared with the present study, which in part may be due to the lower workload, as well as the different mode of exercise compared with the present study (walking vs. cycling). Further, their study is limited as the glucose-fructose beverage was only enriched with ^{13}C - glucose, hence their data is likely underestimated as based solely on exogenous glucose oxidation, not including any fructose contribution. In contrast, Peronnet et al. (9) showed no differences in exogenous glucose oxidation following glucose ingestion ($1.75 \text{ g}\cdot\text{min}^{-1}$) when matching both the relative and absolute exercise intensity between HA and sea level. In comparison the rates of exogenous carbohydrate oxidation were also lower at both HA ($0.43 \text{ g}\cdot\text{min}^{-1}$) and sea level (relative: $0.54 \text{ g}\cdot\text{min}^{-1}$, absolute: $0.50 \text{ g}\cdot\text{min}^{-1}$) compared with the present study. The greater availability of carbohydrate in the present study is due to the likely higher overall carbohydrate absorption rates (36), due to glucose and fructose's distinctly different intestinal transport mechanisms.

Acute hypoxic exposure is associated with reduced insulin sensitivity in both men (37) and women (38) due to increased catecholamine's and cortisol levels. However, overall the hyperglycemia in

both conditions was similar, which is in contrast to studies that have reported elevated plasma glucose concentrations following carbohydrate ingestion during acute hypoxia compared with sea level (9, 29). Furthermore, the plasma glucose concentrations in the present study, stimulated insulin to similar concentrations, despite at least *moderately* higher metanephrine and normetanephrine concentrations at HA. Therefore, the present study does not support the idea of insulin resistance being a plausible explanation for the reduced exogenous carbohydrate oxidation during exercise on acute exposure to HA.

The absolute whole body carbohydrate oxidation was reduced in women at HA compared with sea level. These data are consistent with the previous literature in men (9, 10), following glucose-fructose or glucose ingestion during matched relative exercise intensity. When comparing these data with our previous research in men (10), the reduction in whole body carbohydrate oxidation during 2 hours of exercise at HA is less (~40g vs. ~129g). This may be explained by the lower absolute cycling intensity for the women (105.0 ± 17.2 watts) compared with the men (114.9 ± 9.7 watts), with a lower total energy expenditure (~1154 kcal vs. ~1347 kcal). However, preferential use of fat as a fuel source by women may also be an explanation.

A small difference of 7% in total energy expenditure between HA and sea level is due to the lower absolute workload at HA (105.0 ± 17.2 vs. 114.7 ± 18.9 watts). This will have only marginally contributed to the lower absolute oxidation of whole body carbohydrate at HA and is unlikely to be the full explanation as the magnitude of difference (~24%) far outweighs any difference in total energy expenditure. The primary explanation is that reduced exogenous carbohydrate oxidation makes the most significantly contribution towards the lower whole body carbohydrate oxidation during the second hour of exercise at HA.

In contrast to the literature in men (10, 39), absolute and relative fat oxidation did not significantly increase during exercise at HA compared with sea level. These data are supported by the similar free fatty acid concentrations, suggesting the rate of utilization is comparable despite the higher normetanephrine and metanephrine concentrations. The fact that fat oxidation was not significantly affected by acute exposure to HA in these women, may be due to their higher fat oxidation at sea level (baseline effect) compared with men. This has been ascribed to women having a greater proportion of body fat (12), a greater lipid content in muscles fibers (11), a higher percentage of type 1 muscle fibers (40) and better mobilization of fatty acids from subcutaneous adipose tissue linked to cyclic changes in estrogen and progesterone (13). However, it is interesting to note that the magnitude of fat oxidation in women at HA, was very similar compared with our previous study in men (50% vs. 51%), as was the use of pre-existing muscle glycogen (17% vs. 16%), exogenous carbohydrate oxidation (32% vs. 30%) and glucose release from the liver (3%). This suggests that women and men fuel exercise at HA in a very similar fashion following the ingestion of glucose-fructose.

The sample size in the present study is smaller than originally intended, due to participant attrition. Post-hoc power analysis revealed that the rate of exogenous carbohydrate oxidation provided a power of 57%, 71% and 57% for 60, 90 and 120 minutes of cycling, respectively, using the mean difference and associated standard deviation at each time point and an alpha level of 0.05. Therefore, this study is slightly underpowered compared with the accepted 80% and should be interpreted in this context.

In conclusion, acute exposure to hypoxia reduced the exogenous oxidation of glucose and fructose compared with normoxia during 2 hours of cycling at the same relative exercise intensity. This

may have practical implications, as the conventional carbohydrate ingestion recommendations alter fuel use compared with normoxia. Thus, further research is required to identify an optimal dose for women on acute HA exposure, as well as establishing the mechanism for reduced exogenous carbohydrate oxidation.

Acknowledgements

The authors would like to thank all the participants for their time and effort, as well as Leeds Beckett University for funding this research. We give a special thanks to the extended research team that assisted with data collection, as well as Sandra Small for help with breath ^{13}C CO_2 analysis and Eleanor McKay for help with ^{13}C glucose analysis. The authors declared that the results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by ACSM.

Conflict of Interest

The authors report no conflict of interest.

References

1. Hochachka PW, Stanley C, Matheson GO, McKenzie DC, Allen PS, Parkhouse WS. Metabolic and work efficiencies during exercise in Andean natives. *J Appl Physiol* (1985). 1991;70(4):1720-30.
2. Griffiths A, Shannon OM, Matu J, King R, Deighton K, O'Hara JP. The effects of environmental hypoxia on substrate utilisation during exercise: a meta-analysis. *J Int Soc Sports Nutr*. 2019;16(1):10. Epub 2019/03/01.

- 397 3. Brooks GA, Butterfield GE, Wolfe RR, et al. Increased dependence on blood glucose after
398 acclimatization to 4,300 m. *J Appl Physiol* (1985). 1991;70(2):919-27.
- 399 4. Roberts AC, Reeves JT, Butterfield GE, et al. Altitude and beta-blockade augment glucose
400 utilization during submaximal exercise. *J Appl Physiol* (1985). 1996;80(2):605-15.
- 401 5. Katayama K, Goto K, Ishida K, Ogita F. Substrate utilization during exercise and recovery
402 at moderate altitude. *Metabolism*. 2010;59(7):959-66.
- 403 6. Friedmann B, Bauer T, Menold E, Bartsch P. Exercise with the intensity of the individual
404 anaerobic threshold in acute hypoxia. *Med Sci Sports Exerc*. 2004;36(10):1737-42.
- 405 7. Young AJ, Evans WJ, Cymerman A, Pandolf KB, Knapik JJ, Maher JT. Sparing effect of
406 chronic high-altitude exposure on muscle glycogen utilization. *Journal of applied physiology:*
407 *respiratory, environmental and exercise physiology*. 1982;52(4):857-62.
- 408 8. Bouissou P, Guezennec CY, Defer G, Pesquies P. Oxygen consumption, lactate
409 accumulation, and sympathetic response during prolonged exercise under hypoxia. *Int J Sports*
410 *Med*. 1987;8(4):266-9. Epub 1987/08/01.
- 411 9. Peronnet F, Massicotte D, Folch N, et al. Substrate utilization during prolonged exercise
412 with ingestion of (13)C-glucose in acute hypobaric hypoxia (4,300 m). *Eur J Appl Physiol*.
413 2006;97(5):527-34.
- 414 10. O'Hara JP, Woods DR, Mellor A, et al. A comparison of substrate oxidation during
415 prolonged exercise in men at terrestrial altitude and normobaric normoxia following the
416 coingestion of 13C glucose and 13C fructose. *Physiological reports*. 2017;5(1).
- 417 11. Tarnopolsky MA. Gender differences in substrate metabolism during endurance exercise.
418 *Canadian journal of applied physiology = Revue canadienne de physiologie appliquee*.
419 2000;25(4):312-27.

12. Knechtle B, Muller G, Willmann F, Kotteck K, Eser P, Knecht H. Fat oxidation in men and women endurance athletes in running and cycling. *Int J Sports Med.* 2004;25(1):38-44. Epub 2004/01/30. doi: 10.1055/s-2003-45232.
13. Frontera WR, Suh D, Krivickas LS, Hughes VA, Goldstein R, Roubenoff R. Skeletal muscle fiber quality in older men and women. *American journal of physiology Cell physiology.* 2000;279(3):C611-8.
14. Tremblay J, Peronnet F, Massicotte D, Lavoie C. Carbohydrate supplementation and sex differences in fuel selection during exercise. *Med Sci Sports Exerc.* 2010;42(7):1314-23.
15. M'Kaouar H, Peronnet F, Massicotte D, Lavoie C. Gender difference in the metabolic response to prolonged exercise with [13C]glucose ingestion. *Eur J Appl Physiol.* 2004;92(4-5):462-9.
16. Riddell MC, Partington SL, Stupka N, Armstrong D, Rennie C, Tarnopolsky MA. Substrate utilization during exercise performed with and without glucose ingestion in female and male endurance trained athletes. *Int J Sport Nutr Exerc Metab.* 2003;13(4):407-21.
17. Wallis GA, Dawson R, Achten J, Webber J, Jeukendrup AE. Metabolic response to carbohydrate ingestion during exercise in males and females. *Am J Physiol Endocrinol Metab.* 2006;290(4):E708-15.
18. Beidleman BA, Rock PB, Muza SR, et al. Substrate oxidation is altered in women during exercise upon acute altitude exposure. *Med Sci Sports Exerc.* 2002;34(3):430-7.
19. Lundby C, Van Hall G. Substrate utilization in sea level residents during exercise in acute hypoxia and after 4 weeks of acclimatization to 4100 m. *Acta physiologica Scandinavica.* 2002;176(3):195-201.

- 442 20. Kuipers H, Keizer HA, Brouns F, Saris WH. Carbohydrate feeding and glycogen synthesis
443 during exercise in man. *Pflugers Arch.* 1987;410(6):652-6.
- 444 21. Conkin J. PH₂O and simulated hypobaric hypoxia. *Aviation, space, and environmental*
445 *medicine.* 2011;82(12):1157-8.
- 446 22. Wasserman K, Hansen J, Sue D, Casaburi R, Whipp B. Principles of exercise testing and
447 interpretation: including pathophysiology and clinical applications. 3rd ed. Philadelphia:
448 Lippincott Williams & Wilkins; 1999. p64-68
- 449 23. Morrison DJ, O'Hara JP, King RF, Preston T. Quantitation of plasma ¹³C-galactose and
450 ¹³C-glucose during exercise by liquid chromatography/isotope ratio mass spectrometry. *Rapid*
451 *Commun Mass Spectrom.* 2011;25(17):2484-8.
- 452 24. Craig H. Isotopic standards for carbon and oxygen and correction factors for mass-
453 spectrometric analysis of carbon dioxide. *Geochimica et Cosmochimica Acta.* 1957;12(1-2):133-
454 49.
- 455 25. Peronnet F, Massicotte D. Table of nonprotein respiratory quotient: an update. *Can J Sport*
456 *Sci.* 1991;16(1):23-9.
- 457 26. Craig H. The geochemistry of the stable carbon isotopes. *Geochim Cosmochim Acta.*
458 1953;3:53-92.
- 459 27. Mosora F, Lacroix M, Luyckx A, et al. Glucose oxidation in relation to the size of the oral
460 glucose loading dose. *Metabolism.* 1981;30(12):1143-9.
- 461 28. Peronnet F, Massicotte D, Brisson G, Hillaire-Marcel C. Use of ¹³C substrates for
462 metabolic studies in exercise: methodological considerations. *J Appl Physiol.* 1990;69(3):1047-
463 52.

- 464 29. Young AJ, Berryman CE, Kenefick RW, et al. Altitude Acclimatization Alleviates the
465 Hypoxia-Induced Suppression of Exogenous Glucose Oxidation During Steady-State Aerobic
466 Exercise. *Frontiers in physiology*. 2018;9:830.
- 467 30. Gautier JF, Pirnay F, Jandrain B, et al. Endogenous substrate oxidation during exercise and
468 variations in breath $^{13}\text{CO}_2/^{12}\text{CO}_2$. *J Appl Physiol* (1985). 1993;74(1):133-8.
- 469 31. Trimmer JK, Casazza GA, Horning MA, Brooks GA. Recovery of $(^{13}\text{CO}_2)$ during rest
470 and exercise after $[1-(^{13}\text{C})\text{acetate}]$, $[2-(^{13}\text{C})\text{acetate}]$, and $\text{NaH}(^{13}\text{CO}_3)$ infusions. *Am J Physiol*
471 *Endocrinol Metab*. 2001;281(4):E683-92.
- 472 32. Pallikarakis N, Sphiris N, Lefebvre P. Influence of the bicarbonate pool and on the
473 occurrence of $^{13}\text{CO}_2$ in exhaled air. *Eur J Appl Physiol Occup Physiol*. 1991;63(3-4):179-83.
- 474 33. Peronnet F, Rheume N, Lavoie C, Hillaire-Marcel C, Massicotte D. Oral $[^{13}\text{C}]\text{glucose}$
475 oxidation during prolonged exercise after high- and low-carbohydrate diets. *J Appl Physiol*.
476 1998;85(2):723-30.
- 477 34. Brooks GA. Lactate production under fully aerobic conditions: the lactate shuttle during
478 rest and exercise. *Federation proceedings*. 1986;45(13):2924-9.
- 479 35. Batterham AM, Hopkins WG. Making meaningful inferences about magnitudes.
480 *International journal of sports physiology and performance*. 2006;1(1):50-7.
- 481 36. Shi X, Summers RW, Schedl HP, Flanagan SW, Chang R, Gisolfi CV. Effects of
482 carbohydrate type and concentration and solution osmolality on water absorption. *Med Sci Sports*
483 *Exerc*. 1995;27(12):1607-15.
- 484 37. Larsen JJ, Hansen JM, Olsen NV, Galbo H, Dela F. The effect of altitude hypoxia on
485 glucose homeostasis in men. *The Journal of physiology*. 1997;504 (Pt 1):241-9.

38. Braun B, Horton T. Endocrine regulation of exercise substrate utilization in women compared to men. *Exercise and sport sciences reviews*. 2001;29(4):149-54.
39. Matu J, Deighton K, Ispoglou T, Duckworth L. The effect of moderate versus severe simulated altitude on appetite, gut hormones, energy intake and substrate oxidation in men. *Appetite*. 2017;113:284-92.
40. Glenmark B. Skeletal muscle fibre types, physical performance, physical activity and attitude to physical activity in women and men. A follow-up from age 16 to 27. *Acta Physiol Scand Suppl*. 1994;623:1-47.

Figure Captions

Figure 1. The relative (% of energy yield) contribution of exogenous and endogenous substrate oxidation during the second hour of cycling at high altitude and sea level.

Figure 2. Oxidation rates of exogenous CHO (a), plasma glucose (b), glucose released from the liver (c) and muscle glycogen (d) during the second hour of cycling. * high altitude significantly lower compared with sea level ($P<0.05$).

Figure 3. Plasma glucose (a), plasma lactate (b), serum insulin (c), serum free fatty acids (d), plasma metanephrine (e) and plasma normetanephrine (f) concentrations at rest and during 2 hours of cycling. * high altitude significantly higher compared with sea level ($P<0.05$).

ACCEPTED VERSION